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Effects of nortriptyline on the activities of human and rat liver microsome bufuralol 1′-hydroxylase *in vitro*

TU Zhi-Guang[1], Caroline E SEDDON, Alan R BOOBIS, Donald S DAVIES

(Department of Clinical Pharmacology, Royal Postgraduate Medical School, London W12 OHS, UK)

ABSTRACT The effects of nortriptyline *in vitro* on the activities of optical isomer and racemate bufuralol 1′-hydroxylase in man and Wistar rat liver microsomal fractions were studied. There was a dose-dependent inhibitory effect of nortriptyline on bufuralol 1′-hydroxylase in both species. While the concentration of nortriptyline $\geqslant$0.32 μmol/L and $\geqslant$1.6 μmol/L, the activities of (+), (−) and (±) bufuralol 1′-hydroxylase were significantly reduced in man and rat, respectively. The values of inhibitor concentration causing 50% reduction (IC_{50}) to (+), (−) and (±) bufuralol 1′-hydroxylase were 10, 19 and 14 μmol/L for human and 4, 10, 6 μmol/L for rat, respectively. It was shown by improved Dixon's plot that the inhibitory type was competitive, and the inhibitory constant (K_I) values to (+), (−) and (±) bufuralol 1′-hydroxylase were 5, 3, 4 μmol/L for human and 55, 29, 43 μmol/L for rat, respectively. These results indicate that nortriptyline is a very potent competitive inhibitor to bufuralol 1′-hydroxylase in man and Wistar rat.

KEY WORDS liver microsomes; nortriptyline; cytochrome P-450; adrenergic *beta* receptor blockaders

The debrisoquine 4-hydroxylation has been studied most extensively on human genetic polymorphisms of drug oxidation, with two phenotypes, the extensive metabolizer (EM) and the poor metabolizer (PM)[1]. This polymorphism is due to the defect or functional absence of some form(s) of liver microsome cytochrome P-450 manipulated by the autosomal Mendelian recessive[2,3]. Bufuralol, a non-selective β-adrenoceptor blocker, 1′-hydroxylation and antidepressant drug nortriptyline E-10-hydroxylation in man have been shown to be of genetic polymorphism, and are catalysed by the isozyme of cytochrome P-450 involved in debrisoquine 4-hydroxylation[4-8]. It has been reported that nortriptyline is a potent inhibitor to debrisoquine 4-hydroxylase in man[7]. In present paper we investigated the effects of nortriptyline *in vitro* on the optical bufuralol isomer 1′-hydroxylase activities of hepatic microsomal fraction in man and Wistar rat.

MATERIALS AND METHODS

Liver microsome Six human liver samples were obtained from the renal transplantdonors who died in accidents or were maintained on life support system until the kidneys and livers were removed with the permission of Local Research Ethics Committee. Six ♂ Wistar rats (204±SD 6 g) were purchased from Olac, Biceser, Oxon. Rats were permitted to access food and water freely until 18 h prior to death. Human and rat liver microsomes were isolated by the ultracentrifugation and stored at −80℃ as previously reported[8]. The protein concentration of microsomal fraction was determined with bovine serum albumin fraction *V* as the standard reported before[8].

Chemicals (+), (−) and (±) bufuralol hydrochloride and 1′-hydroxybufuralol were all kindly provided by Roche

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[1] Now in: *Department of Pharmacology, Chongqing University of Medical Sciences, Chongqing 630046, China*

Products. Nortriptyline hydrochloride was purchased from Eli Lilly Co. NADPH (tetrasodium salt, type I) and bovine serum albumin fraction V were purchased from Sigma Co. All solvents were HPLC grade and other reagents were of AR grade.

Bufuralol 1′-hydroxylase activity The liver microsome bufuralol 1′-hydroxylase activity was assayed by the straight phase HPLC-fluorescence spectrometry as published procedures[8] with some modification: Waters Model 510 pump was used instead of twin Altex Model 100 A pumps, and the mobile phase comprised 88% methyl *tert*-butyl ether, 12% acetonitrile and 0.024% perchloric acid. In the dose-dependent inhibition study, bufuralol concentration were 30 and 300 μmol/L in man and rat microsome incubation mixture, respectively. In the inhibitory kinetics study, the high and low concentrations of bufuralol were 30 and 10 μmol/L for human microsome, 300 and 60 μmol/L for rat microsome, respectively. The aqueous solutions of nortriptyline hydrochloride were freshly prepared on the day of use. Pre-experiments had shown that nortriptyline did not interfere the assay of bufuralol 1′-hydroxylase activity. All tests were made in duplicate.

RESULTS

Tab 1 indicated that nortriptyline was a very potent inhibitor to both human and rat hepatic microsome bufuralol 1′-hydroxylase. Even at as low as the concentration of 0.32 μmol/L, nortriptyline significantly inhibited the 1′-hydroxylase activities of the optical isomer and the racemate of bufuralol in man, and at or above the concentration of 1.6 μmol/L, nortriptyline also significantly reduced the rat liver bufuralol 1′-hydroxylase activities of the isomers and racemate. There was a dose-inhibition relationship in both species. The inhibitor concentration causing 50% reduction of bufuralol 1′-hydroxylase activity (IC_{50}) was determined with the graphic procedure as previously described[9]. The values of IC_{50} on human liver microsome bufuralol 1′-hydroxylase activities were 14, 10 and 19 μmol/L for (±), (+) and (−) bufuralol, respectively. With rat liver microsome bufuralol 1′-hydroxylase, the IC_{50} were 6, 4 and 10 μmol/L for (±), (+) and (−) bufuralol, respectively.

Tab 1. Effects of nortriptyline *in vitro* on (+), (−) and (±) bufuralol 1′-hydroxylase activities in human and rat liver microsomes. $n = 6$, $\bar{x} \pm SD$. $^{**}P<0.05$, $^{***}P<0.01$.

Nortriptyline (μmol/L)	Bufuralol 1′-hydroxylase activity (pmol/mg·min) (+)	(−)	(±)
In human liver microsomes			
0	134±20	57±7	85±13
0.32	120±21***	50±9***	77±15**
1.6	107±17***	46±8***	66±9***
8	72±9***	39±5***	51±9***
40	31±7***	20±6***	25±6***
200	13±2***	13±2***	12±4***
In Wistar rat liver microsomes			
0	2060±237	1250±178	1700±310
1.6	1440±300***	1030±102***	1320±212***
8	890±205***	660±107***	770±180***
40	660±200***	530±50***	610±177***
200	460±140***	400±54***	500±180***
1000	200±47***	320±45***	260±48***

The inhibition type of nortriptyline to bufuralol 1′-hydroxylase and the inhibition constant (K_i) were determined with improved Dixon's plot method[10] (Fig 1). It was revealed that nortriptyline was a competitive inhibitor of bufuralol 1′-hydroxylase of the optical isomer and the racemate in both species. The values of K_i of nortriptyline to human liver microsome bufuralol 1′-hydroxylase were 4 μmol/L for (±) bufuralol, 5 μmol/L for (+) bufuralol, and 3 μmol/L for (−) bufuralol. On rat liver microsome bufuralol 1′-hydroxylase, the values of K_i were 43, 55 and 29 μmol/L for (±), (+) and (−) bufuralol, respectively.

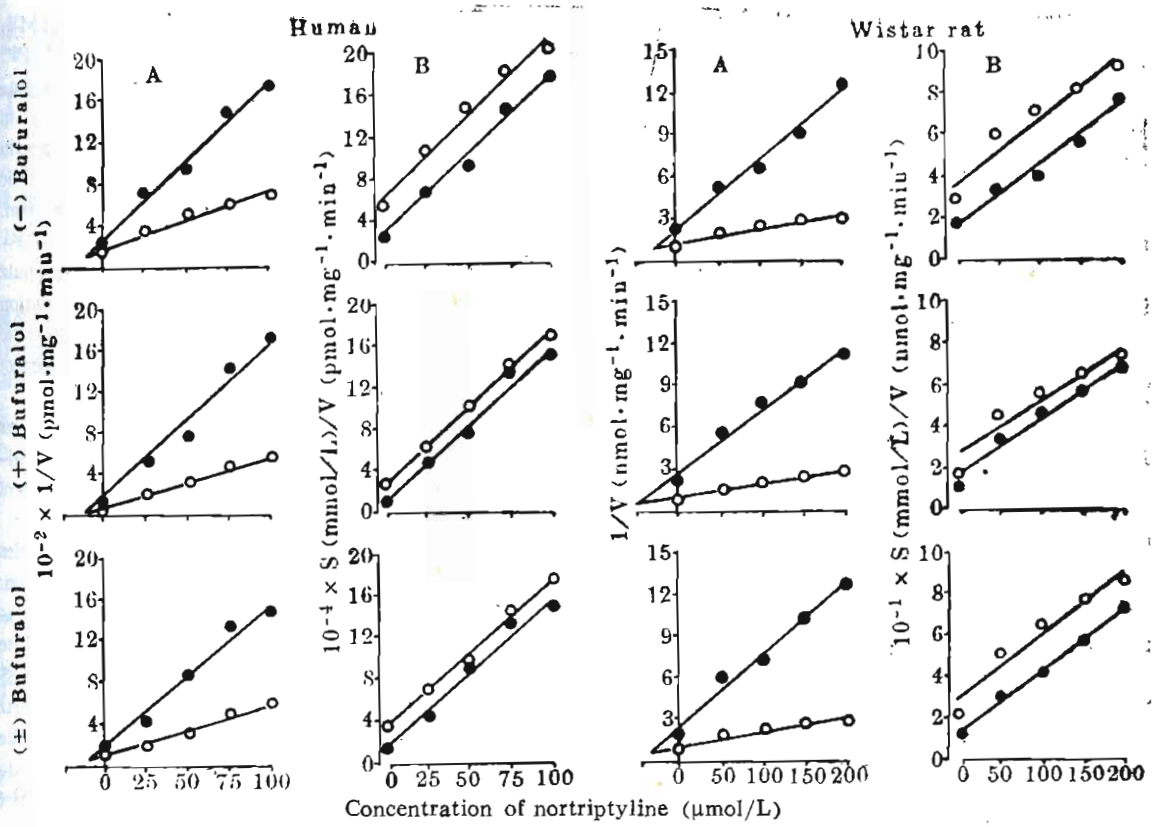

Fig 1. Dixon's (A) and Cornish-Bowden's (B) plots analysing the inhibitory effects of nortriptyline on (−), (+) and (±) bufuralol 1′-hydroxylase activities in human and Wistar rat liver microsomes ($n=4$). The high (○) and low (●) concentrations of bufuralol were 0.03 and 0.01 mmol/L for human, 0.3 and 0.06 mmol/L for Wistar rat, respectively.

DISCUSSION

It has been reported that there is reciprocal competitive inhibition among the compounds which are metabolized by the isozymes of cytochrome P-450 catalysing the 4-hydroxylation of debrisoquine[7,8,11]. Nortriptyline could competitively inhibit the biotransformation of sparteine, of which the oxidation is also involved by the same genetic variation of debrisoquine 4-hydroxylase[12]. Our present study has proved that although like most optical isomers, (+), (−) bufuralol are metabolized by the liver microsome bufuralol 1′-hydroxylase at different velocity *in vitro*, nortriptyline is a very potent competitive inhibitor to (+), (−) bufuralol 1′-hydroxylase in man and Wistar rat. This result is consonant with the observation that debrisoquine is a potent competitive inhibitor of bufuralol 1′-hydroxylase of both optical isomers[8]. However, our finding would call the attention to the possible clinical interaction when bufuralol is administrated simultaneously with nortriptyline.

Quinidine is a very potent competitive inhibitor of human bufuralol 1′-hydroxylase, it could bind to human bufuralol 1′-hydroxylase but is not metabolized by this enzyme[13]. In addition, we have found that the inhibitory effect of quinidine on bufuralol 1′-hydroxylase was of significant species difference in man and wistar rat, while comparing the IC_{50} values, quinidine

was about 480-fold more potent to inhibit human bufuralol 1′-hydroxylase than to that of Wistar rat (to be published). Clearly, nortriptyline, a substrate of bufuralol 1′-hydroxylase, has not shown such species difference in this study, and in contrast, nortriptyline is more potent to inhibit bufuralol 1′-hydroxylase in Wistar rat than to that in man. These findings suggest that the chemical structure or configuration of bufuralol 1′-hydroxylase between man and Wistar rat is probably different, and so quinidine could bind more tightly to human bufuralol 1′-hydroxylase than to that of Wistar rat, but to nortriptyline it is on the contrary. These results warn that it should be careful to extend the observations of drug interaction involved by the genetic polymorphism of debrisoquine 4-hydroxylation with animal model to man.

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